REMARKS

Applicants cancel claims 28, 33 and 34 and amend claims 1-3, 6, 9, 10, 15, 22-24 and 29. New claims 37-41 have been added, therefore claims 1-6, 9, 10, 15, 21-27, 29-32 and 35-41 are pending. New claims 37-39 recite a method for assessing whether a compound enhances the uptake and clearance of a cholesterol-containing low density lipoprotein. Support for this claim can be found within the Specification at page 30, Example 4. New claims 40 and 41 recite a method to determine if a compound causes a change in the structure of apolipoprotein B-100 in a cholesterol-containing low density lipoprotein thus increasing the binding of an epitope on the apolipoprotein B-100 to an LDL-receptor using a two-step sandwich immunoreactivity assay.

Rejection of Claims under 35 U.S.C. § 112

Claims 1-6, 9, 10, 15 and 21-36 are rejected under 35 U.S.C. § 112, second paragraph for failing to particularly point out and distinctly claim the subject matter that Applicants regard as their invention. In response to the Examiner's concerns, Applicants have amended these claims as described below according to the grouping outlined in the Office Action.

Independent Claim 1 With Dependent Claims 2-5, 21 and 22

Claim 1-- The preamble to claim 1 has been amended to support the phrase "low density lipoprotein." Also, the phrase "or other animal" has been deleted. Step c) has been amended to more distinctly claim and define that the compound binds to the cholesterol-containing low density lipoprotein. Applicants have adopted the wording suggested by the Examiner in this

regard. Step d) has been amended to clarify any confusion that existed in relation to the preamble of the claim. This step now recites that the last step in the method of claim is "determining whether the complex results in a change in the binding affinity of the lipoprotein to the low density lipoprotein receptor."

Claims 2, 3 and 22—These claims have been amended to comport with amended claim 1.

Independent Claim 6 With Dependent Claims 10, 23-28 and 30

Claim 6 – Step iii) has been amended as suggested by the Examiner. Step iii) has also been amended to clarify any confusion that may have existed in relation to the preamble of the claim. This step now recites that the last step in the method of claim is "determining whether the complex alters the three dimensional conformation of the low density lipoprotein such that the binding of the low density lipoprotein to a low density lipoprotein receptor is enhanced."

Claims 23, 24, 28 and 30—Claims 23, 24 and 30 have been amended to comport with amended claim 6. Claim 28 has been cancelled.

Independent Claim 9 With Dependent Claims 29 and 33-34

Claim 9—This claim has been amended to clarify the claim language. Steps i) – vi) have been amended to more distinctly recite the steps for the sandwich immunoreactivity assay specifically regarding the detection and quantification steps. Applicants wish the Examiner to note that the detection step has been clarified to include detection of a label. Step vi) has also been amended to clarify the correlation between a change in the structure of Apo B-100 caused by the compound to the amount of LDL captured by the assay.

Claims 33 and 34—These claims have been cancelled.

Independent Claim 15 With Dependent Claims 31, 32, 35 and 36

Claim 15—This claim has been amended to clarify the terms "lipoprotein" and "cholesterol-containing lipoprotein" Both phrases have been amended to recite "cholesterol-containing low density lipoprotein."

Claim 35 and 36—These claims have not been amended but Applicants believe that amendments to claim 15 satisfy the concerns of the Examiner.

Prior Art Rejections

The amended claims are directed to a method to determine whether a compound will increase the clearance of a low density lipoprotein in a host, that includes mixing the compound with low density lipoprotein; determining whether the compound and the low density lipoprotein form a complex; and determining whether the complex alters the three dimensional conformation of the lipoprotein such that the binding of the lipoprotein to a lipoprotein receptor is enhanced. As stated on pages 13-14 of the application, prior to this discovery, it was not known that one could lower serum cholesterol by administering a compound that intercalates into cholesterol-bearing LDL in a manner that increases binding efficiency to clearing receptors. **Since the present claims are assay claims based on this novel mechanism of action, they cannot be rendered obvious by the prior use or disclosure of compounds to lower cholesterol that act through unknown mechanisms and thus cannot and do not teach the public how to select compounds with that activity.**

Rejection of claims 1-6, 9-10, 15 and 21-36 Under 35 U.S.C. § 103(a) as Obvious Over Parthasarathy in view of Koren *et al.*

The Examiner rejects claims 1-6, 9-10, 15 and 21-36 under 35 U.S.C. § 103(a) as obvious in light of U.S. Patent No. 5,262,439 to Parthasarathy in view of U.S. Patent No. 6,107,045 to Koren *et al.* Applicants respectfully traverse this rejection, but would first like to make the Examiner aware that U.S. Patent No. 5,262,439 to Parthasarathy is commonly owned by AtheroGenics, Inc, the assignee of the instant application

The Examiner asserts that Parthasarathy discloses compounds comprising water-soluble probucol derivatives that are administered for use as LDL clearance drugs. This is not true. The '439 patent teaches a method for treating an oxidation related condition in an animal by administering intravenously in an aqueous solution to said animal, a pharmaceutically effective dose of a probucol compound prodrug, said prodrug having hydrolyzable ester substitutes at either or both of the phenyl hydroxy groups of the probucol compound. The patent also suggests that the hydrolyzed probucol compound has hypocholesterolemic properties, i.e., it lowers cholesterol. The '439 patent does not state that the compounds are LDL clearance enhancing compounds as that term is used in the present specification.

Further, incidentally, it has now been discovered in animal testing and confirmed in human clinical trials that monoesters of probucol are not significantly hydrolysed *in vivo*. In fact, in humans, only a few percent of the monosuccinic acid of probucol is hydrolyzed, which is insufficient to impart any independent therapeutic effect. Therefore, the teaching of the '439 patent with regard to the in vivo administration of a probucol compound to obtain the hypocholesterolemic compound probucol is not operative and is misplaced. The '439 patent

discloses or suggests nothing about the biological activity of the monosuccinic acid ester of probucol. Specifically, clinical human trials have established that a representative prototype of monoester of probucol, the monosuccinic acid ester, is not metabolized to probucol in vivo. Applicant administered a single oral dose of the ^{14}C labeled monosuccinic acid ester of probucol to six healthy male volunteers. The radio labeling was in the probucol portion of the molecule, not the succinct acid portion, and so the test would have picked up radio labeled hydrolyzed probucol if it had been formed. The results showed that virtually all of the radioactivity in plasma (94.37 –97.84%) was unhydrolyzed ester. The predominant form of excretion was through the feces, where 85-97 percent of the radioactivity was the unhydrolyzed ester and the remaining radioactivity came from hydroxylated, hydroxy-des-t-butyl, and glucuronidated derivatives. No free probucol was identified.

Thus, Applicant has established that the monosuccinic acid ester of probucol is not a prodrug of probucol, and the biological efficacy of the ester is completely attributable to the parent esterified molecule. Applicant found similar results following oral administration of the ^{14}C labeled ester in both the dog and rat.

Applicant notes that the '439 patent also disclosed that the probucol compounds could be used in *in vitro* environments. The in vitro applications include the preservation of materials in solution. Hydrolysis under these conditions refers to the conversion of the ester into an acid and an alcohol, often catalyzed by an excess of acid or base (for example, by saponification). Saponification is a term which typically refers to a process in which an ester (in this case the monosuccinic acid ester of probucol) is heated with aqueous alkali such as sodium hydroxide to form an alcohol (i.e., probucol). Therefore, the in vitro applications include the possibility of

heat and acid or base to assist in the hydrolysis. Monoesters of probucol are hydrolyzed in laboratory conditions with added acid or base and thus can be used in the '439 in vitro preservation methods. However, these uses are not relevant to the pending claims.

The fact that the monosuccinic acid ester ester of probucol does not hydrolyze in vivo to yield chemically free hydroxyl groups runs counter to the purposes of the '439 patent, and could not have been predicted from the disclosure of the '439 patent.

The Examiner suggests that the '439 patent reference differs from Applicant's invention in failing to disclose isolating LDL and determining the amount of LDL after administration of the probucol compound. As stated above, the '439 doesn't specifically address LDL clearance, and only discloses in vivo activity by the incorrect reference to the use of compounds as prodrugs of probucol. The Examiner indicates that the Koren reference discloses quantifying immunoreactive concentrations of various lipoproteins using sandwich immunoreactive assays, ELISA or polyacrylamide gel electrophoresis. The conclusion reached by the Examiner is that one of ordinary skill in the art at the time the invention was made would have been motivated to use the methods disclosed by Koren to detect the binding to the probucol compounds taught by Parthasarathy.

As the Examiner is aware, to establish a *prima facie* case of obviousness, the following criteria must be met: (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine the reference teachings; (2) there must be a reasonable expectation or success; and (3) the combined references must teach or suggest all the claim limitations. The teaching or suggestion must be found in the prior art and not in the applicant's disclosure. The

references cited by the Examiner do not provide any suggestion or motivation that they can be combined in the manner described in the Office Action. Furthermore, the combined references do not teach or suggest all the claim limitations.

One of ordinary skill in the art would not have been motivated to combine the compounds disclosed in Parthasarathy with the assays of Koren to invent the current screening methods because Parthasarathy provides no teaching or suggestion of the mechanism by which those compounds operate to lower LDL levels. This is a critical point. Understanding a mechanism for a particular class of compounds **is required** to allow one to develop methods to **screen** or **assay** for future candidates that work via the same mechanism. Parthasarathy does not disclose that his compounds might enhance lipoprotein clearance after subsequent binding to a lipoprotein receptor. The Examiner states that "the claimed process is not directed to a new use" but is the same use and method as described by Parthasarathy and that the instant claims "merely recite a newly discovered mechanism." Applicants assert that this interpretation of the instant claims is misplaced. The instant claims are directed to **methods of screening** for compounds which enhance the clearance of cholesterol-containing lipoproteins that act though a **newly** discovered mechanism. The '439 patent is directed to a **method of medical therapy** without any teaching about how to select compounds other than those in the patent based on the novel and fundamental mechanism of action to lower LDL described for the first time in this application.

Since one skilled in the art would not have known the mechanism through which certain esters of probucol were working, there was no basis to select other compounds that would function to lower cholesterol levels by **binding to the cholesterol-containing lipoprotein**.

By similar reasoning, one skilled in the art would not have considered using the assays of Koren because Koren discloses assays directed to **quantifying the amount of lipoproteins present in a sample of blood.** The assays disclosed in Koren are not concerned with, and do not teach, determining alterations in binding to an LDL receptor or with clearance of LDL form the host. There would not have been any motivation to use or modify the assays of Koren to screen for active compounds because the methods of Koren do not teach or suggest anything to screen for other than **blood levels** of lipoproteins.

In summary, neither the '439 patent nor the Koren reference teach or suggest the claimed method of **how to select** a compound that is an LDL clearance enhancing drug.

CONCLUSION

Based on the above-presented amendments and comments, Applicants request that the Examiner allow all pending claims.

Respectfully submitted,

Sherry M. Knowles /with express permission Kristin Chrodzinsky reg# 51,561
Registration No. 33,052

King & Spalding LLP
191 Peachtree Street
Atlanta, Georgia 30303
Telephone: 404-572-3541
Fax: 404 572-5145

MARKED-UP VERSION OF THE CLAIM AMENDMENTS

1. (Three times amended) A method to assess whether a compound first binds to and then enhances the clearing of a cholesterol-containing low density lipoprotein (LDL) after subsequent binding to [the] **a** low density lipoprotein receptor in a host [~~human or other animal~~] **by increasing the binding affinity of the cholesterol-containing low density lipoprotein to the low density lipoprotein receptor, said method** comprising:

(a) administering the compound to the host ;

(b) isolating the cholesterol-containing low density lipoprotein from the host,

(c) determining whether [~~the~~] binding [~~of~~] **has occurred between** the compound [~~to~~] **and** the cholesterol-containing low density lipoprotein **from the host; thus forming** [forms] a complex; and

(d) determining whether the complex results in a change in the [~~three dimensional conformation of the lipoprotein that enhances~~] the binding affinity of the **cholesterol-containing low density** lipoprotein to the low density lipoprotein receptor.

2. (Amended) The method of claim 1, wherein the compound changes the conformation of apolipoprotein in the **cholesterol-containing** low density lipoprotein (LDL).

3. The method of claim 1, wherein the cholesterol-containing **low density** lipoprotein is very low density lipoprotein (VLDL).

4. (Amended) The method of claim 1, wherein the binding of the compound to the cholesterol-containing low density lipoprotein is assessed by a sandwich immunoreactivity assay.

5. The method of claim 1, wherein the binding of the compound to the cholesterol-containing low density lipoprotein is assessed using agarose electrophoresis.

6. (Three times amended) A method to determine whether a compound first binds to and then increases the clearance of a low density lipoprotein after subsequent binding to [~~the~~] **a** low density lipoprotein receptor in a host **by increasing the binding affinity of the low density lipoprotein to the low density lipoprotein receptor, said method** comprising

(i) mixing the compound with **the** low density lipoprotein;

(ii) determining whether the compound binds to the low density lipoprotein and forms a complex; and

(iii) determining whether the complex alters the three dimensional conformation of the **low density** lipoprotein such that the binding of the **low density** lipoprotein to a **low density** lipoprotein receptor is enhanced.

9. (Three times amended) A method to determine if a compound causes a change in the structure of apolipoprotein B-100 in a cholesterol-containing low density lipoprotein **thus increasing the binding of** [, wherein,] an epitope on the apolipoprotein B-100 [binds] to an LDL-receptor, comprising:

(i) mixing the compound with and allowing it to bind to **cholesterol-containing** low density lipoprotein **forming a complex**;

(ii) [~~carrying out a sandwich immunoreactivity assay on~~] **exposing** the [~~compound low density lipoprotein mixture using~~] **complex to** a first **capture** antibody **that is attached to a solid phase material and is** directed to the epitope on apolipoprotein B-100 that binds to the LDL-receptor, **forming a combination;**

(iii) using a second [~~, capture~~] antibody [~~that is attached to a solid phase and~~] which binds to the [~~first antibody~~] **combination**;

(iv) detecting the second [capture] antibody bound to the [~~first antibody~~] **combination by the addition of a third antibody to which is attached a label;**

(v) quantifying the amount of the **captured complex** [~~first antibody LDL low density lipoprotein compound captured by the second antibody~~] **by quantifying the amount of label** ; and

(vi) comparing the amount of [~~LDL~~] **cholesterol-containing** low density lipoprotein captured by the assay to a control**, wherein an increase in the amount of cholesterol-containing low density lipoprotein captured indicates an increased binding to the low density lipoprotein receptor.**.

10. (Twice amended) The method of claim 6, wherein the conformational change in **the low density** lipoprotein is assessed by observing a change in the electrophorectic mobility pattern of the **the low density** lipoprotein using electrophoresis.

15. (Three times amended) A method for assessing whether a compound first binds to a **cholesterol-containing** lipoprotein, enhancing the binding of the **cholesterol-containing** lipoprotein to a low density lipoprotein hepatic receptor and thus lowering plasma cholesterol, the method comprising:

(a) allowing the compound to form a complex with a cholesterol-containing lipoprotein in vivo,

(b) isolating the resulting complex, and

(c) determining whether the formation of the complex causes a change in the three dimensional conformation of apoB-100 in the **cholesterol-containing** lipoprotein that enhances the binding of the lipoprotein to the LDL hepatic receptor.

21. (Amended) The method of claim 2, wherein the apolipoprotein is apoB-100.

22. (Twice amended) The method of claim 1, wherein the **low density** lipoprotein receptor is hepatic .

23. (Twice amended) The method of claim 6, wherein the **low density** lipoprotein is VLDL.

24. The method of claim 6, wherein the **low density** lipoprotein receptor is hepatic.

25. (Amended) The method of claim 6, wherein the determination of whether the compound binds to the low-density lipoprotein and forms a complex is assessed by a sandwich immunoreactivity assay.

26. (Amended) The method of claim 6, wherein the determination of whether the compound binds to the low-density lipoprotein and forms a complex is assessed using agarose electrophoresis.

27. The method of claim 6, wherein the compound alters the conformation of apoB-100.

28. (CANCELLED) The method of claim 6, wherein the lipoprotein receptor is the low density lipoprotein (LDL) receptor.

29. (Twice amended) The method of claim 9, wherein the control is [~~cholesterol-containing~~] low density lipoprotein in the absence of test compound.

30. (Amended) The method of claim 10, wherein the cholesterol-containing low-density lipoprotein is VLDL.

31. (Amended) The method of claim 15, wherein the formation of the complex is determined by a sandwich immunoreactivity assay.

32. (Amended) The method of claim 15, wherein the formation of the complex is determined using agarose electrophoresis.

33. (CANCELLED) (Amended) The method of claim 9, wherein the apolipoprotein is apoB-100.

34. (CANCELLED) (Amended) The method of claim 9, wherein the lipoprotein receptor is a low density lipoprotein (LDL) receptor.

35. (Amended) The method of claim 15, wherein the cholesterol-containing low-density lipoprotein is LDL.

36. (Amended) The method of claim 15, wherein the cholesterol-containing low-density lipoprotein is VLDL.